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RESEARCH PAPER

An Insulin Molecularly Imprinted Electrochemical Sensor Based on Epitope Imprinting

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Abstract: A new molecularly imprinted electrochemical sensor for direct detection of insulin was prepared based on epitope imprinting. The molecularly imprinted polymer was fabricated by electropolymerization with C-terminal polypeptide of insulin self-assembled on the Au electrode as template molecule and *o*-PD as functional monomer. After elution of template molecules by NaOH solution, the imprinting cavities were formed with the three-dimensional structure matching with the C-terminal polypeptide of insulin molecule. The imprinting cavities could specifically recognize and rebind with insulin molecules. With $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a probe, the insulin could be indirectly detected. There was a linear relationship between the response current and the concentration of insulin in the range of 1.0×10^{-14}–5.0×10^{-13} M, and the detection limit was 7.24×10^{-15} M ($3\sigma/S$). The developed sensor exhibited good selectivity and stability; and was successfully applied to determination of insulin in serum samples with satisfactory results.

Key Words: Insulin; Molecular imprinting; Electrochemical sensor; Epitope imprinting

1 Introduction

Insulin is one of the most important hormone regulating metabolism of carbohydrate and fat *in vivo*[1], such as promoting the synthesis of glycogen, fattiness and protein, affecting the growth and development of human cells, and it is important for human's health. At present, the detection methods of insulin include enzyme-linked immunosorbent assay (ELISA)[2], electrochemiluminescence immunoassay[3], radioimmunoassay[4] and chromatography[5,6]. However, these methods have the drawbacks of using expensive reagents, complex operations and time-consuming procedure in detection of insulin. Therefore, it is necessary to develop rapid identification methods with high specificity to insulin.

In rescent years, molecularly imprinted sensors have been developed rapidly because of their good specificity[7], recognition ability and simple manipulation, and were widely used in the detection of large molecules such as proteins[8–10]. For example, Prasad *et al*[11] prepared a molecularly imprinted polymer (MIP) modified electrode by free radical polymerization on the surface of multi-walled carbon nanotubes (MWCNTs–$CH=CH_2$) with insulin as template molecule, (*p*-acryloylaminophenyl)-{(4-aminophenyl)-diethyl ammonium}-ethylphosphate as monomer and ethylene dimethacrylate as cross-linker. This electrode was used for insulin determination by measuring the irreversible oxidation peaks at 0.8 V produced by the tyrosine residue in insulin. However, the template molecule of the MIP was difficult to elute due to the complicated conformation and large steric hindrance effect of insulin. Besides, the lower electrochemical activity of insulin would lead to the unsatisfactory sensitivity, and the excessive oxidation potential made the determination of insulin suffering from severe interference from reducing substance in samples.

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Epitope imprinting[12–14] is a new protein imprinting method developed in recent years. With polypeptides in surface of protein as template molecules, this method uses the formed imprinting cavities to specifically recognize target polypeptide and protein[15,16], which overcomes the limitation that the template molecules embedded in MIP by normal imprinted method is difficult to be eluted. Epitope imprinting used to prepare insulin molecular imprinting sensor has not been reported yet. In this study, C-terminal polypeptide of insulin (C-insulin polypeptide) as template molecule was directly self-assembled on the surface of Au electrode, and then molecularly imprinted membrane was prepared by electrochemical polymerization with *o*-phenylenediamine (*o*-PD) as functional monomer. After elution of the C-insulin polypeptide, the imprinted cavities formed and were used to specifically recognize the target peptides. With $[Fe(CN)_6]^{3-/4-}$ as electrochemical probe, the MIP was used for the indirect determination of insulin by measuring the redox current of the probe on the electrode.

2 Experimental

2.1 Apparatus and reagents

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were carried out on CHI600D work station (Shanghai Chenhua Instrument Co., Ltd., China), and electrochemical impedance spectroscopy (EIS) was measured on an Autolab work station (Metrohm Co., Ltd., Netherland). All of the electroanalytical measurements were completed with a standard three-electrode system, which consisted of an Ag/AgCl reference electrode, a platinum wire electrode as auxiliary electrode and an Au electrode as the working electrode. The pH of solution was measured by PHS-3C model pH meter (Shanghai Leici Instruments, China).

Carbinol and *o*-PD were purchased from China National Pharmaceutical Group Chemical Reagent Co., Ltd.. $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, KCl, $Na_2HPO_4{\bullet}12H_2O$ and $NaH_2PO_4{\bullet}2H_2O$ were purchased from Xilong Chemical Co., Ltd., China. The reagents used in the experiment were analytical pure, and ultra-pure water were used in the experiment.

C-insulin polypeptide solution (1.0×10^{-4} M) and *o*-PD solution (5 mM) was prepared with phosphate buffer solution (PBS, 0.02 M, pH 7.4) as solvent.

2.2 Experimental methods

2.2.1 Self-assembly of C-insulin polypeptide

Appropriate amount of C-insulin polypeptide solution was added in a 0.5 mL centrifuge tube. Then a polished Au electrode was immersed in the C-insulin polypeptide solution for 1 h for self-assembly. With $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a probe, the sensor were characterized by CV, DPV and EIS.

2.2.2 Preparation of MIP sensors and non- MIP (NMIP) sensors

Figure 1 shows the preparation process of insulin molecularly imprinted electrochemical sensor based on epitope imprinting. Using Au electrode self-assembled with C-insulin polypeptide as working electrode and *o*-PD solution as supporting solution, electro-polymerization was performed to obtain molecularly imprinted membrane by cyclic scan for 10 cycles at a scanning speed of 50 mV s^{-1} in the potential range of 0–0.8 V. With NaOH solution (2 M) as eluent, the modified electrode was eluted for 35 min under magnetic stirring. The molecularly imprinted sensor was successfully prepared after the template molecule being eluted. The NMIP sensor was prepared by the same method of MIP sensor but without the self-assembly of C-insulin polypeptide.

2.2.3 Electrochemical measurements

The preparation process of the sensors was characterized by CV and DPV with $[Fe(CN)_6]^{3-/4-}$ solution (5 mM) as electroactive probe. The potential range of DPV was from –0.2 V to +0.8 V and amplitude was 50 mV. The potential range of CV was from –0.2 V to +0.6 V and scanning rate was 50 mV s^{-1}. The potential of EIS was 0.19 V, with frequency range of 100 mHz to 100 kHz and alternating voltage of 5 mV.

3 Results and discussion

3.1 Preparation of molecularly imprinted membrane

The polymerization of molecularly imprinted membrane was performed according to the methods described in Section 2.2.2, and the results are shown in Fig.2. During the electro-polymerization process, the current gradually decreased and tended to a stable value, indicating that an

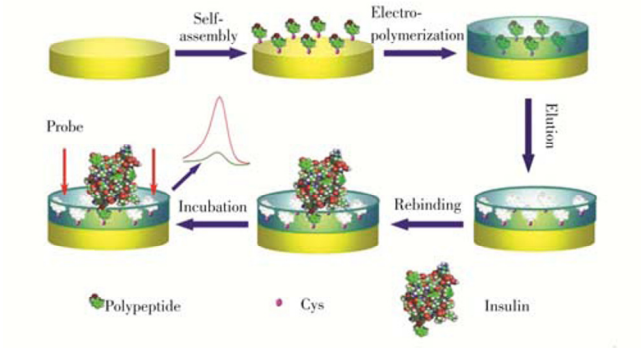

Fig.1 Schematic illustration of fabrication of insulin molecularly imprinted electrochemical sensor based on epitope imprinting